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Antimicrobial Activity of Some Plant Extracts and Their Applications in Homemade Tomato Paste and Pasteurized Cow Milk as Natural Preservatives

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Abstract: Synthetic chemical preservatives are widely used in the food industry to delay the deterioration caused by microbial growth, enzyme activities and oxidation reactions. The last few decades have witnessed marked interest in finding natural food preservatives due to the potential health damage of synthetic preservatives; consumers have become skeptical of consuming foods containing these additives. Polyphenols used as natural preservatives that can be extracted from fruits, vegetables, herbs and spices provide the best alternative for partial or complete replacement of their synthetic analogues. The present study's emphasis was on employing different plant extracts to be efficiently used as antimicrobial agents for developing replacements for the synthetic chemical additives in food products. The study also investigated the antimicrobial potentialities of five medicinal plants, widely used in Egypt (sumac, tamarind, rosemary, roselle and lemon) against six microbial markers (*E. coli*, *P. aeruginosae*, *B. subtilis*, *S. aureus*, *Penicillium* sp. and *A. niger.*). Sumac extracts showed the best activity against all tested microorganisms, producing the widest inhibition zones ranging from 14 to 45 mm, followed by tamarind and roselle extracts, with inhibition zones ranging from 8–36 and 8–34 mm, respectively. On the other hand, extracts of rosemary and lemon showed variable antimicrobial activity. All extracts from all tested plants were less active against fungal species than bacterial species. In all cases, the organic extracts (80% methanol, 80% ethanol) showed the same or greater activity than the aqueous extracts. In addition, the methanolic extracts showed the strongest and broadest spectrum. The most sensitive strain to plant extracts was *B. subtilis*, while the most resistant strain was *P. aeruginosae.* The MIC and MBC or MFC values of methanolic extracts were assayed using the broth dilution method. Sumac extract showed the best activity against all tested microorganisms with the lowest values of MIC and MBC or MFC (from 0.260 to 0.877 and 0.310 to 1.316 mg/mL, respectively, for bacteria, and from 1.975 to 2.5 and 2.5 to 4.444 mg/mL, respectively, for fungi). Interestingly, the tested extracts inhibited microbial growth in tomato paste and pasteurized cow milk for a long storage period (increase shelf life) as compared to the control samples. In conclusion, herbal and spice extracts could be successfully applied as natural antimicrobials for the elimination of food borne microbes and pathogen growth.

Keywords: food spoilage microbes; foodborne pathogens; antimicrobial activity; plant extracts; natural preservatives

1. Introduction

Spoilage microorganisms grow in food and result in the production of undesirable flavors or odors, changing texture or appearance, and the loss of nutritional values of the food products. These undesirable changes make the product not suitable for human consumption. Many microorganisms can cause food spoilage, such as *Bacillus*, *Pseudomonas*, *Lactobacillus*, and some molds [1,2]. Fungi are a major cause of food deterioration and spoilage worldwide, ranking second to insects [3,4]. Foodborne pathogenic microorganisms may cause diseases in humans after consumption. For example, *Bacillus cereus*, which produces emetic and diarrheal toxins causes diseases, emetic syndrome and diarrhea, and the main food source of infection are rice, pasta, noodles and pastry [5]; *Campylobacter coli* and *Campylobacter jejuni*, which produce cytolethal distending toxin, cause campylobacteriosis, and the main food sources of infection are poultry products and unpasteurized milk [6]; *Clostridium botulinum*, which produces botulinum toxin, causes botulism, with the main food sources of infection being improperly processed canned foods [5]; *Escherichia coli* O157:H7, which produces shiga-toxin, causes hemorrhagic colitis, and the main food sources of infection are ground meats, raw or under-pasteurized milk and sprouts [5,7]; *Listeria monocytogenes,* which produces listeriolysin O, causes listeriosis, with the main food sources of infection being soft cheeses from unpasteurized milk and ready-to-eat products [7]; *Salmonella Typhi*, *Salmonella Typhimurium* and *Salmonella Enteritidis*, which produce enterotoxins, cause typhoid fever and salmonellosis (gastroenteritis), and the main food sources of infection are any type of food: meat, poultry, fish, milk, eggs, vegetables, water, etc. [5,7]; and *Staphylococcus aureus*, which produces heat stable enterotoxins, causes gastrointestinal symptoms, with the main food sources of infection being meat, dairy products and salads [5]. Sometimes, the growth of pathogenic organisms may not change the quality and sensory properties of the food. Therefore, the contamination of pathogens may not be detected without performing microbiological tests [8,9]. According to the WHO Initiative to Estimate the Global Burden of Food-borne Diseases, 31 global hazards caused 600 million food-borne illnesses and 420,000 deaths in 2010 [10,11]. Currently, the commonly used food preservatives are synthetic or artificial chemicals; however, there are concerns regarding the use of these compounds. Firstly, they might be harmful to human health. For example, nitrite, which is used as a curing agent to inhibit *Clostridium* growth in meat products, can react with amines and ammonium compounds to form nitrosamines, which are carcinogenic [12,13]. The most commonly used preservatives are sodium benzoate, sulfur dioxide, nitrites, sorbic acid, propionic acid, and sodium and potassium nitrates [14]. They are used within the permissible limits organized by The Codex Alimentarius and the European legislation on food additives [15]. The assessment of food additives worldwide is supported by the control system of the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Expert Committee on Food Additives [16]. There are also many ways to preserve food, including traditional techniques such as freezing, boiling, curing, canning, pickling and many more, as well as modern techniques such as freeze drying, pasteurization, irradiation, pascalization, vacuum packing, biopreservation, modified atmosphere hurdle and technology [17]. Secondly, their effectiveness is highly related to the conditions of the foods, such as moisture content, pH and the oxidation-reduction potential of the food. Thirdly, "natural" is the new trend in the food industry. Artificial food preservatives are not preferred by consumers who want natural foods [18,19]. Hence, the search for newer, safer and more potent antimicrobials is a pressing need [20–29].

Herbs have received a lot of attention as a source of antimicrobial compounds because they are considered time-tested and relatively safe for human use and the environment, and can be applied to food without any problems [30–32]. Plants are rich in a wide variety of secondary metabolites (for protection against aggressor agents, especially microorganisms) such as tannins, terpenoids, alkaloids, coumarins, iridoids, lignans, steroidals, saponins, xanthones and flavonoids, which have been found to have antimicrobial properties [33–35]. *Rhus coriaria* L. (sumac) is a member of the genus Rhus, which contains over 250 individual species of flowering plants and belongs to the family Anacardiaceae [36–38]. *R. coriaria*, which grows wild in the region from the Canary Islands through the Mediterranean region to Iran and Afghanistan, is commonly used as a spice by grinding the dried fruits with salt, which is used as a condiment and sprinkled over kebabs and grilled meat, as well as over salad, and is also widely used as a medicinal herb, particularly for the treatment of indigestion, anorexia, diarrhea, hemorrhagia and hyperglycemia, animals bites and poisons and sexual diseases [39,40]. The fruit of sumac is a novel source of natural antimicrobial and antioxidant agents for the food and pharmaceutical industries [41]. Sumac has significant effects in preventing gram-positive and gram-negative pathogenic bacteria. Previous studies have shown that essential oil and extracts of sumac leaf and fruit have appropriate antimicrobial effects against bacilli, staphylococci, enterococci and lactobacilli [42,43]. *Tamarindus indica* L. (tamarind) belongs to the dicotyledonous family Fabaceae and subfamily Caesalpiniceae [44]. Tamarind has been used for centuries as a medicine plant; its fruits are the most valuable part, which have often been reported as curative in several pharmacopoeias, and the leaves have been proven to have protective activity associated with the presence of polydroxylated compounds, with many of them of a flavonoid nature [45]. Leaf and fruit extract of *Tamarindus indica* showed antibacterial activity against clinical isolates of *Escherichia coli* and *Shigella* [46]. Roselle (*Hibiscus sabdariffa* L.) has been shown to have various bioactivities with therapeutic benefits. These bioactivities are due to the different kinds of phytochemicals present, which include anthocyanins, phenolic acids and flavonoids [47,48]. The roselle water and ethanol extracts showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The inhibition of the roselle ethanol extract against *B. subtilis* and *S. aureus* was slightly higher than that of water extract, but this difference was not significant [49]. Moreover, roselle extracts showed antibacterial activity against bacteria obtained from food or other foodborne pathogens [50,51]. *Rosmarinus officinalis* L. (rosemary) belongs to the Lamiacea family and is popular as a spice and medicinal plant in many countries. It has antibacterial, antifungal, anti-cancer, anti-diabetic, anti-inflammatory, analgesic, antioxidant and endemic effects [52–54]. Carnosic acid and rosmarinic acid may be the main bioactive antimicrobial compounds present in rosemary extracts. From a practical point of view, rosemary extract may be a good candidate for functional foods, as well as for pharmaceutical plant-based products [55,56]. Lemon (*Citrus limon* L.) contains many bioactive compounds such as carotenoids, limonoid, flavonoids, tannin, and terpenoids, which have antibacterial and antioxidant properties [57,58]. Lemon species have antimicrobial activity against different Gram-positive, Gram-negative and yeast pathogens [59]. The main aim of this study was to examine the antimicrobial activity of ethanolic, methanolic and water extracts of sumac, tamarind, rosemary, roselle and lemon against six common food pathogens and spoilage microorganisms, so that new food preservatives can be explored and developed.

2. Materials and Methods

2.1. Isolation and Identification of Tested Microorganisms

Different specimens of spoilage tomato fruit were collected and screened for the presence of food spoilage and foodborne pathogenic microorganisms on nutrient agar medium (Oxoid, Hampshire, UK; for bacteria) and Sabouraud dextrose agar (Oxoid, Hampshire, UK; for fungi) according to Jay et al., 2008 [60] and Adams and Moss, 2000 [61]. The purified bacterial cultures were identified and confirmed after investigating morphological and biochemical characters according to standard laboratory methods reported and recommended

by Bergey's Manual of Systematic Bacteriology [62–64]. Colonies representative of each type of bacterium were stained by the Gram method, then examined microscopically for Gram staining reaction (positive staining purple or negative staining pink), size (small, medium, or large) and shape (coccobacilli, rods or cocci). Further characterization of the isolates was done using conventional biochemical tests (oxidase, catalase, methyl red test, indole production, citrate utilization, the Voges–Proskauer test, triple sugar iron and coagulase tests), following Markey et al., 2013 [65].

The unknown isolated fungi were identified based on macro and micro morphology, reverse and surface coloration of colonies, and the slide culture technique [66,67]. Four of the most common bacterial species were selected, including two Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)*; in addition, two common fungal species were selected (*Aspergillus niger* and *Penicillium* sp.).

2.2. Plant Extracts Preparation

Five common herbs and spices in Egypt were selected based on previous literature or the publicity in the Egyptian market (Table 1), and were purchased from local markets in different Egyptian regions. The samples were dried in an oven at 50 °C to a constant moisture content, then powdered. Next, 100 g of every dried powdered plant material was soaked with 500 mL of 80% methanol or 80% ethanol or distilled boiled water (for preparing the infusion extract and for preparing the decoction extract, 100 g of every powdered plant was decocted in 500 mL distilled water for 30 min) separately in a sterile conical flask for 48 h with continuous shaking. Then, the samples were filtered through 4 layers of muslin cloth and centrifuged for 10 min. The supernatant was collected and filtered with Whatman filter paper No. 2. The obtained extract was concentrated using a rotary evaporator (SBW-1, Shanghai Shenbo Instrument Co., Shanghai, China) under reduced pressure at 45 °C to eliminate the solvent. The residual fraction was freeze-dried (lyophilized). A section of each powdered extract was diluted to 10 mg/mL using 10% dimethyl sulfoxide (DMSO) as solvent (stock solutions), and then sterilized by filtration through a bacterial filter of pore size 0.45 µm using positive pressure. Then, filtrate was kept at 4 °C in refrigerator until use [37,68,69].

Table 1. A list with the Latin names, English names, local names and used parts of the medicinal plants tested.

Family	Latin Name	English Name	Local Name	Part Used
Anacardiaceae	*Rhus coriaria*	Sumac	Sumac	Fruits
Fabaceae (Leguminosa)	*Tamarindus indica*	Tamarind	Tamrhindy	Pods
Lamiaceae (Labiatae)	*Rosmarinus officinalis*	Rosemary	Rosemary	Aerial parts
Malvaceae	*Hibiscus sabdariffa*	Roselle	Karkadae	Red calyces
Rutaceae	*Citrus limon*	Lemon	Limoon	Fruits

2.3. Determination of Total Phenolic Compounds (TPC)

The total phenolic content (TPC) of the plant extracts was determined by Folin–Ciocalteu assay using gallic acid as the standard according to Kaur and Kapoor, 2002 [70], with few modifications. Briefly, 100 µL of different concentrations of test sample was mixed with 1 mL of diluted FC reagent (1:10). After 10 min, 1 mL of 7.5% (*w*/*v*) sodium carbonate solution was added to the mixture and incubated in the dark for 90 min. The absorbance was recorded at 725 nm. The phenolic content was calculated from a calibration curve and expressed as gallic acid equivalents (mg GAE/g DW).

2.4. Determination of Radical Scavenging Activity (RSA)

The method developed by Brand-Williams et al., 1995 [71], was used for the measurement of DPPH radical scavenging activity. The extracted solution (0.1 mL) at concentration 400 ppm was mixed with 3.9 mL of 0.075 mM DPPH. The mixture was left in the dark

at room temperature for exactly 30 min. The blank was made by replacing the extracted solution with methanol (0.1 mL). The absorbance of DPPH purple-colored solution at 517nm was measured using a spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = 1 - (\text{Abs sample} - \text{Abs blank})/\text{Abs control} \times 100$$

2.5. Antimicrobial Assay

The antimicrobial activity of selected extracts were determined using the disc diffusion method according to Black and Black, 2018 [72], Thiem and Goslinska, 2004 [73] and Arokiyaraj, 2013 [74]. Mueller Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungi) were sterilized by autoclaving at 121 °C for 15 min, cooled, poured into Petri dishes and inoculated with the selected isolates by striking the swab over the surface of the medium in three directions to confirm a complete distribution. Sterile filter paper discs (Whatman No. 3, 6 mm diameter and three layers) were saturated by stock solutions of 100 µL of each extract (10 mg/mL); the disks were allowed to dry for one hour, then placed on the surface of inoculated plates. The used organic solvents and distilled water disks served as negative controls. The plates were kept in a refrigerator for one hour to allow better diffusion of the extract prior to incubation at 37 °C/24 h for bacteria and 30 °C/96 h for molds. After incubation, the inhibition zones formed around disks were measured in millimeters (including the diameter of the disk (6 mm)). Each experiment was run in triplicates and the means were calculated.

2.6. MIC and MBC or MFC of Methanolic Extracts Determination

The methanolic extracts were selected because they showed the best antimicrobial activity. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by the broth dilution technique in Mueller Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi. In tubes, two-fold serial dilutions of each methanolic extract were made from the diluted stock solution, using broth as diluent, to obtain concentrations ranging from 0.173 to 10 mg/mL. Each tube was inoculated with the tested organism (at a concentration of 10^8 cells/mL for bacteria and 10^6 spores/mL. for fungi; 24 h age). With each group, tubes of uninoculated medium with and without extract were included to act as a control to ensure sterility and clarity of the medium. A third control tube containing inoculated medium but without extract was also included to ensure the ability of the organism to grow in the medium. All the tubes were incubated at 37 °C/24 h for bacteria and 30 °C/72 h for molds, and examined for turbidity as an indicator of microbial growth. The MIC is defined as the lowest concentration that inhibits a visible growth in liquid media. One hundred microliters (µL) were taken from each MIC concentration, as well as other MIC concentrations, and introduced onto MHA or SDA to determine the MBC and MFC values, respectively. The plates were incubated at 37 °C/24 h for bacteria and 30 °C/72 h for molds. MBC or MFC were defined as the concentration at which the microorganism fails to grow in broth in the presence of inhibitor and fails to grow when broth is plated onto agar in the absence of the inhibitor, respectively [75,76].

2.7. Application of Ethanolic Extracts in Homemade Tomato Paste

Tomato was purchased from a local market, washed, cut to small pieces and mixed with water (1:2 *w*/*v*); then, the salt was added (2%), and the mixture was crushed in blender and then overheated at 80–100 C° with continuous steering until the desired texture was reached. The obtained paste was divided into sterile screw-capped glass bottles. Each extract and sodium benzoate (which is the most widely used preservative in food) was added (0.03%) individually and mixed with the tomato paste. The treated samples and the control were stored at two different temperatures (room temperature and refrigerator (4 °C)), and examined every four days for the appearance of bacteria or fungi [77–79].

2.8. Application of Ethanolic Extracts in Raw Cow Milk

The raw cow's milk was divided into 6 equal parts: the first part was left without any treatment as a comparison sample, the other five parts were treated at a rate of 3000 ppm with the ethanolic extracts of sumac, tamarind, rosemary, roselle and lemon, respectively. This concentration was selected based on the concentration used for the preservative sodium benzoate, which was 0.03% or 3000 ppm. The concentration was microbiostatic, when 3 g of the lyophilized extract were dissolved in a liter of solvation solution. All treatments were incubated at a temperature of 25 °C for 6 h, and then tested for total microbial and coliform count.

2.8.1. Total Microbial Count

Total microbial counts of untreated raw cow milk and samples treated with ethanolic extracts of sumac, tamarind, rosemary, roselle and lemon (3000 ppm) were determined at room temperature (25 ± 2 °C) after 6 h. Plate count agar medium (PCA, Oxoid, Hampshire, UK) was used [80], and the plates were incubated for 48 h at 30 °C. Total microbial count was calculated directly in colony forming units (CFU mL^{-1}).

2.8.2. Count of Coliform Bacteria

Coliform bacteria were enumerated in untreated raw cow milk and treated with ethanolic extracts of sumac, tamarind, rosemary, roselle and lemon (3000 ppm). Coliform count was determined at room temperature (25 ± 2 °C) after 6 h, and calculated directly in colony forming units (CFU mL^{-1}) using violet red bile agar (Oxoid, Hampshire, UK). The plates were incubated for 48 h at 37 °C.

2.9. Application of Ethanolic Extracts in Pasteurized Cow Milk

Neutralized and filter sterilized extracts (3000 ppm) were added to 10 mL of pasteurized cow milk (72 °C, 5 min). Milk samples were stored at 4 °C and at room temperature (25 ± 2 °C) for 20 days. Bead formation was observed on a microscope slide surface daily, according to Abdalla et al., 2007 [81].

2.10. Statistical Analysis

Data were expressed as mean ± SD using one-way analysis of variance (ANOVA), followed by the least significant difference (L.S.D.) test. Statistic version 9 was performed for analyses of the data [82]. The differences between the means of the treatments were considered significant ($p < 0.05$) when they were more than the LSD at the 5% levels. All measurements were used in triplicate and statistically analyzed.

3. Results and Discussion

3.1. Total Phenol Content and % DPPH Inhibition of the Plant Extracts

No single method is adequate for evaluating the antioxidant capacity of foods, since different methods can yield widely diverging results. Several methods based on different mechanisms must be used, including a way to measure DPPH radical scavenging activity. According to the results presented in Table 2, sumac extract contained higher TPC and produced higher % DPPH inhibition, with 284.28 (mg of gallic acid/g) and 90.24%, respectively. These results agree with those previously reported by Fereidoonfar et al., 2019 [83]. In addition, tamarind extract contained 196.84 (mg of gallic acid/g) and resulted in 88.60% % DPPH inhibition, respectively, and these present results agree with those previously reported by Santos et al., 2020 [84]. Roselle extract exhibited the results of 29.68 (mg of Gallic acid/g) and 85.24%, respectively; these results agree with those previously reported Purbowati and Maksum, 2019 [85]. Rosemary extract exhibited 17.60 (mg of Gallic acid/g) and 82.72%, respectively; these results agree with those previously reported by Afonso et al., 2013 [86]. Finally, lemon exhibited 14.96 (mg of Gallic acid/g) and 80.16%, respectively; these results agree with those previously reported Sir Elkhatim et al., 2018 [87].

Table 2. Total phenol content (TPC) and % DPPH inhibition of plant extracts.

Plant Extract	TPC mg GAE/g DW	% DPPH Inhibition
Rhus coriaria	284.28 ± 12.6	90.42 ± 25
Tamarindus indica	196.84 ± 8.4	88.60 ± 1.8
Rosmarinus officinalis	17.60 ± 3.5	82.72 ± 2.3
Hibiscus sabdariffa	29.68 ± 2.8	85.24 ± 2.7
Citrus limon	14.96 ± 1.9	80.16 ± 1.4

3.2. Prevalence of Bacteria and Fungi Isolated from Spoilage Tomato Fruit

Seven species of bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*) and five fungi (*Mucor* spp., *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium* spp. and *Penicillium* spp.) were isolated and characterized. The most isolated bacterium was *Bacillus subtilis*, at 36%, while the most isolated fungus was *Mucor* ssp., at 34%. These present results are in agreement with those reported by Bello et al., 2016 [88], who isolated eight species of bacteria and six species of fungi, and reported that the most isolated fungus was *Mucor* ssp., at 28%, while the most isolated bacterium was Bacillus subtilis, at 30%.

3.3. Antimicrobial Activity of Plant Extracts by the Disc Diffusion Method

The susceptibility of selected foodborn spoilage and pathogenic microorganisms towards extracts from five medicinal plant species was tabulated in Table 3, based on their inhibition diameter on agar plates. Sumac extracts showed the best activity against all tested microorganisms, producing the widest inhibition zones ranging from 14 to 45 mm, followed by tamarind and roselle extracts, with inhibition zones ranging from 8 to 36 mm and 8 to 34 mm, respectively. On the other hand, extracts of rosemary and lemon showed variable antimicrobial activity; alcoholic extracts of rosemary exhibited good activity, while aqueous ones showed weak or no activity. Furthermore, all extracts from lemon exhibited very good activity against bacterial species, but weak or no activity against fungal species. All extracts from all tested plants were less active against fungal species than bacterial species. The results showed that the extracts were more active against *B. subtilis* and *S. aureus* (Gram-positive), while less active against others, such as *E. coli* and *P. aeruginosa* (Gram-negative bacteria). The results are in agreement with previous studies which indicated that plant extracts were more active against Gram-positive bacteria than those that are Gram-negative [89–92]. These differences may be attributed to the fact that the cell wall in Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multi-layered structure, bounded by an outer cell membrane, and quite complex [93,94]. The different percentages of microbial growth inhibition can be attributed to the different chemical compositions and modes of action of these plant extracts [95,96]. Moreover, plant-derived flavonoids, phenolic acids, tannins and stilbenes can inhibit the growth and activity of many microorganisms, including bacteria, fungi and protozoa [97–99], as these compounds inhibit the extracellular enzymes or induce the permeabilization and destabilization of the plasma membrane [100]. Plant phenols are effective against drug-resistant pathogens [100]. The activity of decoction extracts was slightly reduced (Table 3), suggesting that the active components of aqueous extracts were not destroyed at high temperatures (heat stable), even with the 30 min treatment at 100 °C. Many extracts from medicinal plants have been reported to possess antimicrobial effects and are used for the purpose of food preservation and for medicinal purposes [31,32,101,102]. In this study, aqueous and organic extracts from the same plant showed different activities. There are no common rules for this, but in most cases, the organic solvent extracts showed the same or greater activity than the aqueous extracts. It was observed that alcoholic extracts of most samples showed the best antimicrobial activities in contrast to aqueous extracts; this may be because the ethanol and methanol solvents are known to have the ability to isolate

more antimicrobials from plants, including anthocyanins, tannins, polyphenols, terpenoids, saponins, xanthoxyllines, totarol, quassinoids, lactones, flavones and phonons, while the water solvent extracts could contain only anthocyanins, starches, tannins, saponins, terpenoids, polypeptides and lectins [33,92,103].

Table 3. Antimicrobial activity of plant extracts by the disc diffusion method.

		Inhibition Zone (mm) *						
Plant Species	**Sol.**	***E. coli***	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***Penicillium* spp.**	***A. niger***	***LSD***
Citrus limon	E	23 ± 2^{c}	18 ± 1^{d}	27 ± 2^{a}	25 ± 1^{b}	0 ± 0^{f}	8 ± 1^{e}	1.60
	M	26 ± 2^{c}	22 ± 2^{d}	34 ± 2^{a}	31 ± 2^{b}	0 ± 0^{f}	9 ± 1^{e}	2.68
	WD	$15 \pm 1^{a,b}$	13 ± 1^{b}	16 ± 1^{a}	$14 \pm 1^{a,b}$	0 ± 0^{c}	0 ± 0^{c}	2.53
	WI	19 ± 2^{c}	11 ± 1^{d}	23 ± 2^{a}	21 ± 1^{b}	0 ± 0^{e}	0 ± 0^{e}	1.46
Hibiscus sabdariffa	E	12 ± 2^{d}	15 ± 2^{c}	31 ± 2^{a}	26 ± 3^{b}	11 ± 1^{d}	$13 \pm 2^{c,d}$	2.31
	M	14 ± 1^{c}	13 ± 1^{c}	34 ± 2^{a}	28 ± 2^{b}	13 ± 1^{c}	12 ± 1^{c}	2.78
	WD	24 ± 1^{b}	25 ± 2^{b}	30 ± 2^{a}	30 ± 2^{a}	10 ± 1^{c}	9 ± 1^{c}	1.15
	WI	22 ± 2^{c}	27 ± 2^{b}	33 ± 3^{a}	32 ± 2^{a}	9 ± 1^{d}	8 ± 1^{d}	2.78
Rhus Coriaria	E	35 ± 2^{c}	28 ± 1^{d}	41 ± 2^{a}	38 ± 2^{b}	19 ± 1^{f}	24 ± 2^{e}	1.55
	M	37 ± 3^{b}	31 ± 2^{c}	45 ± 3^{a}	42 ± 2^{a}	23 ± 2^{d}	26 ± 3^{d}	3.10
	WD	20 ± 2^{b}	24 ± 2^{a}	26 ± 3^{a}	19 ± 2^{b}	14 ± 1^{c}	18 ± 2^{b}	3.11
	WI	24 ± 1^{c}	27 ± 2^{b}	32 ± 2^{a}	31 ± 2^{a}	16 ± 1^{e}	20 ± 2^{d}	2.92
Rosmarinus officinalis	E	15 ± 1^{b}	8 ± 1^{d}	17 ± 1^{a}	13 ± 1^{c}	7 ± 1^{e}	7 ± 1^{e}	0.58
	M	18 ± 1^{b}	$10 \pm 1^{c,d}$	22 ± 2^{a}	18 ± 1^{b}	8 ± 1^{e}	11 ± 1^{c}	2.96
	WD	8 ± 1^{b}	8 ± 1^{b}	13 ± 2^{a}	11 ± 2a	0 ± 0^{c}	0 ± 0^{c}	2.16
	WI	9 ± 2^{b}	10 ± 2^{b}	15 ± 2^{a}	10 ± 2^{b}	0 ± 0^{c}	0 ± 0^{c}	2.36
Tamarindus indica	E	30 ± 2^{bc}	28 ± 1^{c}	34 ± 2^{a}	31 ± 2^{b}	13 ± 2^{d}	15 ± 2^{d}	2.47
	M	29 ± 3^{b}	25 ± 2^{c}	36 ± 3^{a}	35 ± 2^{a}	16 ± 2^{d}	18 ± 3^{d}	3.05
	WD	20 ± 2^{d}	32 ± 2^{a}	26 ± 2^{c}	29 ± 2^{b}	9 ± 1^{f}	12 ± 1^{e}	2.31
	WI	22 ± 1^{c}	31 ± 2^{a}	28 ± 2^{b}	27 ± 2^{b}	8 ± 1^{e}	13 ± 1^{d}	2.78

* Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference. Inhibition zones include the paper disc diameter (6 mm); values calculated as means of triplicates. Sol.: solvent; E: ethanol extract; M: methanol extract; WD: water decoction extract; WI: water infusion extract; 0: no inhibition zone.

The methanolic extracts, in general, gave the maximum size of inhibition zones against all microorganisms. These results confirmed the substantiation of previous studies, which have reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other organic solvents and water [104,105]. On the other hand, Mahasneh and EL-Oqlah, 1999 [106], showed that butanol extracts have superior antimicrobial activity compared with other ones. Another previous study [107] reported that the aqueous extracts were more active against bacteria compared with ethanol and ethyl acetate extracts. Furthermore, another study [108] concluded that the activity is mainly concentrated in the butanol and aqueous extracts. These results agreed with several studies reported previously [43,46,48,54,58] that the sumac, tamarind, roselle, rosemary and lemon extracts had a broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria.

3.4. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanolic Extracts

Tables 4 and 5 show the MIC and MBC or MFC values of methanolic extracts against the selected bacterial and fungal species, respectively, using the broth dilution method. The results of growth of different microbial strains at various incremental levels of extract reflect a clearer picture of the inhibitory effect of selected extracts. Sumac extract showed the best activity against all tested microorganisms, with the lowest values of MIC and MBC or MFC (from 0.260 to 0.877 and 0.310 to 1.316 mg/mL, respectively, for bacteria

and from 1.975 to 2.5 and 2.5 to 4.444 mg/mL, respectively, for fungi). *Bacillus subtilis* was clearly found to be the most sensitive, demonstrating a MIC and MBC of 0.260 to 1.250 and 0.310 to 1.975 mg/mL, respectively. Conversely, Gram-negative species were found to be more resistant than the Gram-positive species, with *Pseudomonas aeruginosae* being the most resistant bacteria, surviving up to 4.444 mg/mL. Fungal species survived at the highest concentrations (Table 4). The results are in accordance with the findings of the disc diffusion assay (Table 3). *Pseudomonas* spp. and *Bacillus* spp. are known to show consistently high resistance to plant antimicrobials; moreover, *Bacillus* spp. were reported to exhibit high sensitivity [109,110].

Table 4. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extracts (mg/mL).

	E. coli		*P. aeruginosa*		*B. subtilis*		*S. aureus*	
Plant Species	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Rhus coriaria	0.585 ± 0.2^{c}	0.625 ± 0.2^{c}	0.877 ± 0.1^{c}	1.316 ± 0.3^{c}	0.26^{d}	0.31 ± 0.07^{d}	0.39 ± 0.2^{c}	0.39 ± 0.1^{c}
Tamarindus indica	$0.625 \pm 0.3^{b,c}$	$0.877 \pm 0.4^{b,c}$	1.316 ± 0.2^{c}	$1.975 \pm 0.5^{b,c}$	$0.39 \pm 0.08^{c,d}$	0.39 ± 0.1^{d}	$0.625 \pm 0.1^{b,c}$	1.25 ± 0.1^{b}
Citrus limon	1.25 ± 0.5^{b}	1.316 ± 0.3^{b}	1.975 ± 0.4^{b}	2.5 ± 0.8^{b}	$0.585 \pm 0.2^{b,c}$	$0.625 \pm 0.2c$	0.877 ± 0.2^{b}	1.975 ± 0.4^{ab}
Hibiscus sabdariffa	$0.877 \pm 0.2^{b,c}$	1.25 ± 0.5^{b}	1.975 ± 0.3^{b}	2.962 ± 0.7^{b}	0.625 ± 0.1^{b}	0.877 ± 0.1^{b}	0.877 ± 0.4^{b}	1.975 ± 0.2^{ab}
Rosmarinus officinalis	2.5 ± 0.3^{a}	2.962 ± 0.2^{a}	2.962 ± 0.2^{a}	4.444 ± 0.85^{a}	1.25 ± 0.4^{a}	1.975 ± 0.2^{a}	1.316 ± 0.2^{a}	2.5 ± 0.3^{a}
LSD	0.659	0.537	0.631	0.993	0.215	0.199	0.367	0.825

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference.

Table 5. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of methanolic extracts (mg/mL).

	Penicillium **spp.**		*Aspergillus niger*	
Plant Species	**MIC**	**MFC**	**MIC**	**MFC**
Rhus coriaria	2.5 ± 0.92^{d}	4.444 ± 1.04^{c}	1.975 ± 0.94^{c}	2.5 ± 0.72^{c}
Tamarindus indica	2.962 ± 0.74^{d}	4.444 ± 1.06^{c}	2.5 ± 0.78^{c}	4.444 ± 0.95^{b}
Hibiscus sabdariffa	4.444 ± 1.02^{c}	6 ± 1.3^{b}	2.962 ± 0.66^{c}	5 ± 1.2^{b}
Rosmarinus officinalis	6.666 ± 1.05^{b}	10 ± 1.0^{a}	5 ± 1.01^{b}	10 ± 1.3^{a}
Citrus limon	$>10 \pm 0.96^{a}$	$>10 \pm 1.2^{a}$	6.666 ± 1.0^{a}	10 ± 1.2^{a}
LSD	1.25	1.52	1.25	1.42

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference.

3.5. Effect of the Extracts on the Keeping Quality of Homemade Tomato Paste

Despite the effectiveness of methanolic extracts against microbial activity and their high content of phenolic compounds, there is great concern regarding their use in foods, as many studies have indicated the toxicity of methanolic extracts on some organs of experimental animals, such as the liver and kidneys [111–113]. As a result, the ethanolic extracts were chosen for further research. Results in Table 6 show the days required for bacterial and fungal growth development in tomato paste samples with or without extracts or sodium benzoate under storage conditions. It was found that extracts and sodium benzoate inhibit microbial growth for a long period of storage time (increase shelf life) compared to control samples. Sodium benzoate was more active against fungi than bacteria, while ethanolic extracts were more active against bacteria than fungi. Sumac extracts exhibit antifungal activity similar to the effect of sodium benzoate, and with a stronger effect than sodium benzoate against bacteria. In addition, the samples stored in the refrigerator were better than that stored at room temperature. This is due to the reduction in microbial physiological activities under low temperatures [60,114]. Antimicrobial action mechanisms of plant extracts and their natural components may be related to: degradation of the cell wall; damage to cytoplasmic membrane and membrane proteins; leakage of intracellular

contents; coagulation of cytoplasm; and interference with active transport or metabolic enzymes—all of which can cause cell death [115,116].

Table 6. Days required for appearance of microbial growth in the homemade tomato paste samples under different storage conditions.

	Fungi		Bacteria	
Samples	**Room Temperature**	**Refrigeration**	**Room Temperature**	**Refrigeration**
Control	4 ± 2^{e}	8 ± 1^{f}	12 ± 2^{e}	16 ± 2^{d}
Sodium benzoate	28 ± 3^{a}	40 ± 3^{a}	24 ± 1^{b}	36 ± 2^{b}
Rhus coriaria	24 ± 3^{b}	32 ± 2^{b}	32 ± 2^{a}	44 ± 3^{a}
Tamarindus indica	16 ± 2^{c}	28 ± 3^{c}	24 ± 2^{b}	36 ± 2^{b}
Hibiscus sabdariffa	16 ± 2^{c}	24 ± 2^{c}	20 ± 1^{c}	24 ± 2^{c}
Rosmarinus officinalis	12 ± 3^{c}	16 ± 2^{d}	16 ± 1^{d}	24 ± 2^{c}
Citrus limon	8 ± 2^{d}	12 ± 2^{e}	24 ± 2^{b}	32 ± 3^{b}
LSD	3.49	3.68	2.85	5.05

Means followed by different small letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference.

3.6. Effect of the Extracts on the Total Bacterial and Coliform Count of Raw Cow Milk

The use herbs and their extracts in preserving milk and its products is not so preferable because they affect the flavor of milk; however, milk was chosen for this study as an applied example of the ability of herbal extracts to reduce the microbial content of milk, and thus, for the rest of the foods that can use these extracts during preservation. Milk is a unique food that contains all the elements necessary for life. It is considered one of the closest foods to the complete food model, as it contains all the basic components of nutrition, namely proteins, carbohydrates, fats, minerals, and vitamins. These compounds are found in a dissolved or suspended state in an abundant amount of water and in quantities appropriate to the body's need for them, and in a way that facilitates the body's use of them [117,118]. Milk is also considered an environment suitable for the growth and activity of all microorganisms, as a result of it containing more than 80% water, and the pH of milk is close to neutrality (6.6–6.8). In addition, it contains the nutrients necessary for the growth and activity of microorganisms [119,120]. Results in Table 7 illustrate the total bacteria counts and coliforms in raw cow milk and cow milk treated with ethanolic sumac, tamarind, roselle, rosemary and lemon extracts. The results showed that total bacterial and coliform counts in untreated raw milk were 4.2×10^6 and 1.9×10^3 CFU mL^{-1}, and increased to 9.2×10^8 and 1.8×10^6, respectively, after 6 h of incubation at 25 °C. Addition of 3000 ppm sumac, tamarind, rosemary, roselle and lemon ethanolic extracts to raw cow milk resulted in reduction of total bacterial count to 4.7×10^3, 5.9×10^3, 2.2×10^4, 8.6×10^4 and 1.8×10^5, whereas coliform growth was completely inhibited. The results in this study indicated that sumac, tamarind, rosemary, roselle and lemon ethanolic extracts reduced the aerobic plate and coliform counts of raw milk. Sumac extract was the most effective in reducing the total bacterial and coliform counts in treated raw milk, followed by the raw milk sample treated with tamarind extract, then the raw milk sample treated with roselle extract, followed by the raw milk sample treated with rosemary, and the finally the raw milk sample treated with lemon extract. The effect is due to the difference in the contents of anti-microbial substances, such as phenolic acids, between the different extracts. These observations are in line with the results presented in Table 1, which show a higher content of total phenolic content and antioxidant activity in sumac extract than the other extracts, followed by tamarind extract, and then roselle extract, rosemary extract, and finally lemon extract. These results are also in line with the results shown in Table 3, which prove that sumac extract had the most effect on microbial activity, followed by tamarind extract, roselle extract, rosemary extract, and finally lemon extract. These present results

agreed with the results of several previous studies [43,46,48,54,58], which reported that the sumac, tamarind, roselle, rosemary and lemon extracts had a broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria.

Table 7. Effect of sumac, tamarind, rosemary, roselle and lemon ethanolic extracts on the total microbial and the coliform count of raw cow milk after 6 h of incubation at 25 °C.

Samples	Total Microbial Count (CFU mL^{-1})	Total Coliform Count (CFU mL^{-1})
Control	9.2×10^{8}	1.8×10^{6}
Rhus Coriaria (Sumac)	4.7×10^{3}	ND
Tamarindus indica (Tamarind)	5.9×10^{3}	ND
Hibiscus sabdariffa (Roselle)	2.2×10^{4}	ND
Rosmarinus officinalis (Rosemary)	8.6×10^{4}	ND
Citrus limon (Lemon)	1.8×10^{5}	ND

ND = not detected.

3.7. Effect of the Extracts on the Keeping Quality of Pasteurized Cow Milk

Results indicated that untreated pasteurized cow milk samples deteriorated after 4 and 7 days of storage at 25 ± 2 °C and 4 °C, respectively. Conversely, treating pasteurized milk with different ethanolic extracts increased its shelf life compared to untreated pasteurized milk, where beads were not formed in treated milk samples after 20 days of storage at both temperatures. As a result of the unhealthy conditions for the production and handling of raw milk, the lack of cooling facilities, and the long period of time between milk production and its delivery to a dairy plant in Egypt, the keeping quality of pasteurized milk produced under Egyptian conditions decreases [81]. Thus, sumac, tamarind, rosemary, roselle and lemon ethanolic extracts could be added to pasteurized milk to provide a subsequent shelf life extension. These findings are also in line with the present results shown in Table 3, which prove that sumac extract had the most effect on microbial activity. Previous studies [31,32,102] also reviewed the safety of some herbal and spice extracts as food additives for extending the shelf-life of a variety of food and dairy products.

4. Conclusions

Microbial spoilage and oxidative reactions reduce the shelf life of foods; therefore, synthetic chemical preservatives have been used in foods to maintain food safety and quality. The present results indicated that the sumac, tamarind, rosemary, roselle and lemon ethanolic extracts can be used as natural preservatives in food as an alternative to artificial preservatives. The application of the medicinal plant extracts in the food industry not only facilitates antimicrobial activities, but also contributes to pharmacological activities such as food antioxidants, healthcare, and the increase in the shelf-life of food products, as well as of food nutrients. Clearly, it is a natural food additive with considerable market prospects.

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